Research Note

A New Kinetic Model for Thermal Inactivation of Microorganisms: Development and Validation Using *Escherichia coli* O157:H7 as a Test Organism[†]

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ABSTRACT

A new kinetic model has been proposed to simulate the nonlinear behavior of survivor curves frequently observed in thermal inactivation of microorganisms. This model incorporates a time component into the first-order inactivation kinetics and is capable of describing the linear, convex, and concave survivor curves. The model was validated using *Escherichia coli* O157:H7 as a test microorganism. Ground beef (93% lean) samples inoculated to 10^7 to 10^8 CFU/g of meat were subjected to immersion heating at 55, 57.5, 60, 62.5, and 65°C, respectively, in a water bath. All the survivor curves in this study showed upward concavity. Linear and nonlinear regressions were used to fit the survivor curves to the linear first-order inactivation kinetics and the proposed model. Analyses showed that the new kinetic model provides a much better estimate of the thermal inactivation behavior of *E. coli* O157:H7 in ground beef.

For decades, the first-order reaction kinetics has been the primary model for quantitatively describing thermal inactivation of microorganisms in foods *(13)*. The fundamental assumption of this kinetic model is that the rate of destruction is a linear function of cell concentration under isothermal conditions. Two basic equations (equations 1 and 2) have been adopted by the food industry to describe and calculate thermal inactivation of microorganisms. The D-value, commonly known as decimal reduction time, is the time needed to destroy 90% or 1 log cycle of microorganisms in a population at a constant temperature. The z-value is the increase in temperature required to reduce the D-value by 90%.

$$\log_{10}(C) = \log_{10}(C_0) - \frac{t}{D} \tag{1}$$

$$\log_{10}(D) = \log_{10}(D_0) - \frac{T}{z} \tag{2}$$

Although the first-order reaction kinetics has been successfully applied in the food industry to calculate and design thermal processes for decades, deviations from this kinetic model have been repeatedly observed by many researchers. Depending on the microorganisms and environmental conditions, four types of survivor curves (Fig. 1) have been reported *(2, 10, 14)*. According to Pflug and Holocome *(11)*, approximately two-thirds of the semiloga-

rithmic survivor curves of homogeneous cultures of microorganisms are not straight curves, and the use of the traditional calculated D-values would be misleading. As a result, overcooking is needed to reduce microorganisms to a safe level for consumption.

The objective of this research was to develop a kinetic model that could more accurately describe the inactivation behavior of microorganisms during thermal processing. Our assumption was that the rate of thermal inactivation is a function of both cell concentration and heating time. *Escherichia coli* O157:H7 was selected as a test pathogen for study because of its association with several serious outbreaks in ground beef *(3, 4)*. Development of an accurate thermal inactivation model is of great interest for consumers who demand fresh, juicy, tender hamburgers, minimum heating, and avoidance of *E. coli* O157:H7-related foodborne poisoning.

MATERIALS AND METHODS

New model development. Traditional first-order kinetics (equation 3) assume that the rate of thermal inactivation decreases linearly with the viable cell concentration in a population under isothermal conditions. Incorporating a time component into equation 3, the rate of thermal inactivation becomes a function of both time and the viable cell concentration (equation 4). A new kinetic model (equation 5) can be obtained after rearranging and integrating equation 3. This resultant model is theoretically more versatile than the first-order kinetic model. Depending on the value of the index *a*, this model is capable of simulating different shapes of inactivation curves. In the special case where $a = 0$, the model reduces to first-order kinetics. If $a > 0$, the semilogarithmic inactivation curve shows downward concavity. At $a < 0$, an upwardly concaved inactivation curve can be obtained.

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† Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.
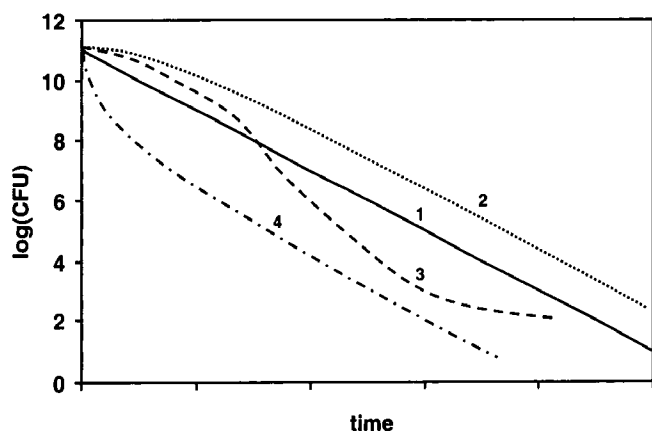
FIGURE 1. *Schematic thermal inactivation survivor curves. Curve 1: First-order thermal inactivation kinetics; curve 2: Convex survivor curve; curve 3: Thermal inactivation with both initial lag and tail phases; and curve 4: Concave or continuously decreasing death rate curve.*

$$\frac{dC}{dt} = -kC \qquad (3)$$

$$\frac{dC}{dt} = -k_T t^a C \qquad (4)$$

$$\ln(C) = \ln(C_0) - \frac{k}{a+1} t^{a+1} \qquad (5)$$

Converting the natural base in equation 5 to the logarithm of base 10, a more convenient form of equation 6 can be derived.

$$\log_{10}(C) = \log_{10}(C_0) - \frac{k \log_{10}(e)}{a+1} t^{a+1} \qquad (6)$$

Denoting

$$A = \log_{10}(C_0), \qquad B = \frac{k \log_{10}(e)}{a+1}, \qquad \alpha = a + 1$$

Equation 6 can be modified into a simplified form.

$$\mathrm{Log}_{10}(C) = A + Bt^\alpha \qquad (7)$$

Ground beef. Ground beef (93% lean) was obtained from a local grocery store. The meat was divided into 50-g portions in plastic bags, sealed, and kept frozen ($-18°C$) until use (<2 months). Prior to experimentation, a 50-g plastic bag was taken out of the freezer, immersed in a 2-liter beaker, and thawed with running water.

Organisms. Four strains of *E. coli* O157:H7 were used in this study: EDL-931, 45753-35, C1-9218, and 933 *(7)*. These strains were obtained from the culture collection of the Microbial Food Safety Research Unit at the Eastern Regional Research Center of the U.S. Department of Agriculture-Agricultural Research Service (Wyndmoor, Penn). *E. coli* O157:H7 strains were previously preserved in 10% glycerol and maintained at $-80°C$. The bacterial strains were propagated and properly maintained with their purity periodically checked and validated with Vitek Test Cards (Gram-Negative Identification Card Plus, or GNI+) using the Vitek Automated Microbiology System (Model Vitek 60, bioMerieux, Inc., Hazelwood, Mo.).

Inoculum preparation. A 0.1-ml portion from each culture was transferred to 10 ml of brain heart infusion broth (Difco Laboratories, Detroit, Mich.) in a 15-ml centrifuge tube and then incubated for 18 h at 37°C. Each stationary-phase culture was centrifuged (5,000 × *g*, 15 min) at 4°C and washed in 0.1% peptone water (wt/vol) twice. Bacterial cell pellets were suspended in 10 ml of 0.1% peptone water to a larger cell level of 10^8 to 10^9 CFU/ml. A 5-ml aliquot from each strain was combined and mixed to prepare a four-strain bacterial cocktail.

Sample preparation, inoculation, and storage. Samples of ground beef (3 g) were aseptically weighed in plastic bags (7 by 15 cm) modified from sterile filter bags (Model SFB 520, Spiral Biotech, Bethesda, Md.). Each sample was inoculated with 0.1 ml of the *E. coli* O157:H7 cocktail to a final cell concentration of 10^7 to 10^8 CFU/g of ground beef. Samples were manually mixed in the bags to ensure even distribution of the bacterial cells. Plastic bags containing meat samples were compressed to a final thickness of 1 to 2 mm and heat sealed after excluding as much air as possible *(7)*.

Thermal inactivation. Samples, separated from one another by approximately 8 mm, were placed in a preheated metal rack.
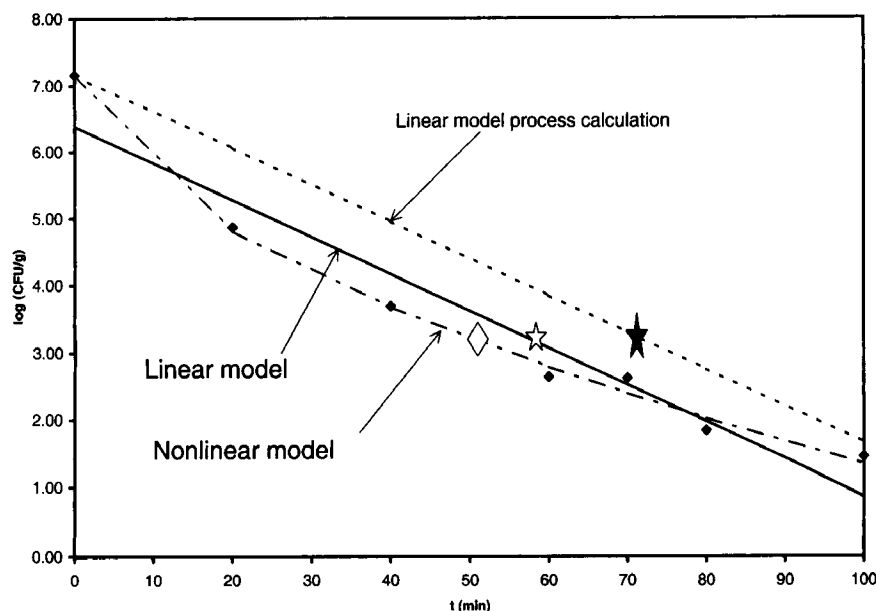

FIGURE 2. *Survivor curve of* E. coli *O157:H7 at 55°C. Thermal inactivation calculated based on the initial cell concentration using the semilogarithmic linear models will be underestimated. Open diamond: 4-D reduction determined by experiment; open star: 4-D reduction using the* D-value *and intercept determined by linear regression; closed star: 4-D reduction calculated from equation 1 using the* D-value *and initial cell load; closed diamond: raw data.*

TABLE 1. *Parameters of linear and nonlinear models at different heating temperatures*

Temperature (°C)	Linear model				Nonlinear model			
	Initial log$_{10}$ (CFU/g)	Intercept log$_{10}$ (CFU/g)	D (min)	R^2	A	B	α	R^2
55.0	7.14[a] ± 0.04[b]	6.45 ± 0.24	17.86 ± 1.24	0.946	7.13 ± 0.12	−0.378 ± 0.062	0.594 ± 0.034	0.993
55.0	7.15 ± 0.04	6.35 ± 0.27	18.48 ± 1.46	0.930	7.15 ± 0.13	−0.445 ± 0.077	0.555 ± 0.037	0.992
57.5	8.29 ± 0.04	6.65 ± 0.41	6.21 ± 0.75	0.829	8.29 ± 0.23	−1.637 ± 0.241	0.391 ± 0.039	0.978
57.5	7.64 ± 0.28	6.54 ± 0.33	6.21 ± 0.61	0.883	7.66 ± 0.30	−0.999 ± 0.249	0.515 ± 0.066	0.961
60.0	8.00 ± 0.01	7.11 ± 2.22	2.19 ± 0.12	0.957	7.91 ± 0.18	−1.235 ± 0.156	0.644 ± 0.044	0.988
60.0	8.09 ± 0.08	6.64 ± 0.38	2.58 ± 0.33	0.835	8.06 ± 0.23	−2.670 ± 0.258	0.409 ± 0.044	0.977
62.5	8.48 ± 0.01	7.34 ± 0.31	1.52 ± 0.13	0.910	8.42 ± 0.33	−2.064 ± 0.367	0.526 ± 0.070	0.962
62.5	8.44 ± 0.11	6.98 ± 0.42	1.90 ± 0.20	0.866	8.55 ± 0.34	−2.316 ± 0.368	0.469 ± 0.055	0.969
65.0	7.29 ± 0.06	6.66 ± 0.32	0.82 ± 0.07	0.948	7.36 ± 0.26	−2.304 ± 0.306	0.629 ± 0.075	0.975
65.0	7.32 ± 0.08	7.03 ± 0.28	0.79 ± 0.06	0.927	7.21 ± 0.39	−1.526 ± 0.407	0.886 ± 0.152	0.952

[a] Mean.
[b] Standard error.

TABLE 2. *Parameters for equations 5 and 6*

Temperature (°C)	a	k
55.0	−0.406 ± 0.034	0.517 ± 0.017
55.0	−0.445 ± 0.037	0.569 ± 0.020
57.5	−0.609 ± 0.039	1.474 ± 0.049
57.5	−0.485 ± 0.066	1.185 ± 0.063
60.0	−0.356 ± 0.044	1.832 ± 0.050
60.0	−0.591 ± 0.044	2.515 ± 0.068
62.5	−0.474 ± 0.070	2.500 ± 0.105
62.5	−0.531 ± 0.055	2.502 ± 0.093
65.0	−0.371 ± 0.075	3.338 ± 0.112
65.0	−0.114 ± 0.152	3.114 ± 0.186

These were then fully immersed in a temperature-controlled circulated water bath (Model Ex-251-HT, Neslab Instruments, Newington, N.H.) and stabilized at 55.0, 57.5, 60.0, 62.5, and 65.0°C. Come-up time was set as 15 s based on previous experiments and computer simulations. At different time intervals depending on temperature, duplicate samples were removed from the water bath, immediately plunged into an ice-water bath, and analyzed within 30 min. All of the experiments were replicated at least twice for each temperature treatment.

Enumeration of surviving bacteria. Sterile 0.1% peptone water was mixed with each sample at a 1/1 (vol/wt) ratio and pummeled for 1 min with a Stomacher (400 lab blender, Tekmar, Cincinnati, Ohio). Serial dilutions were made and plated onto tryptic soy agar (Difco) plates using the spiral plater mentioned above. After 120 min of resuscitation at room temperature to allow for recovery of heat-damaged cells *(9)*, the tryptic soy agar plates were overlaid with 10 ml sorbitol MacConkey agar (Oxoid, Hampshire, UK) pretempered to 47°C. After agar solidification, samples were incubated at 35°C for 48 h. Typical *E. coli* O157:H7 colonies were counted after incubation. An average of two plate readings was used to represent the number of bacteria surviving after heat treatment. This value was used in determining *D*-values with initial bacterial loads included in the linear regression.

Determination of *D*-values and *z*-values. Linear regression was used to determine the slopes of the log$_{10}$(CFU)/g versus time curves using Number Cruncher Statistical Systems—NCSS 2000, which is a comprehensive 32-bit Windows-based statistical analysis package *(5)*. *D*-values were calculated from the slopes of the linear curves (equation 1). The *z*-value of *E. coli* O157:H7 in 93% lean ground beef was determined from the slope of the log$_{10}$(*D*)-temperature plot by linear regression analysis (equation 2).

Nonlinear regression and statistical analysis of experimental data. Nonlinear regression of thermal inactivation data was performed using NCSS 2000 to obtain the parameters of equations 6 and 7. Statistical comparisons were conducted using SAS *(12)*. For nonlinear regression analyses, pseudo R^2 values were constructed by NCSS 2000 to approximate the usual R^2 values used in linear regression *(5)*.

RESULTS AND DISCUSSION

The mean initial cell concentration (log$_{10}$ (CFU)/g) in the ground beef was 7.64 with a standard deviation of ±0.53. All of the survivor curves are upwardly concaved, indicating that the rates of thermal inactivation were higher at the initial stage, then gradually declined under isothermal
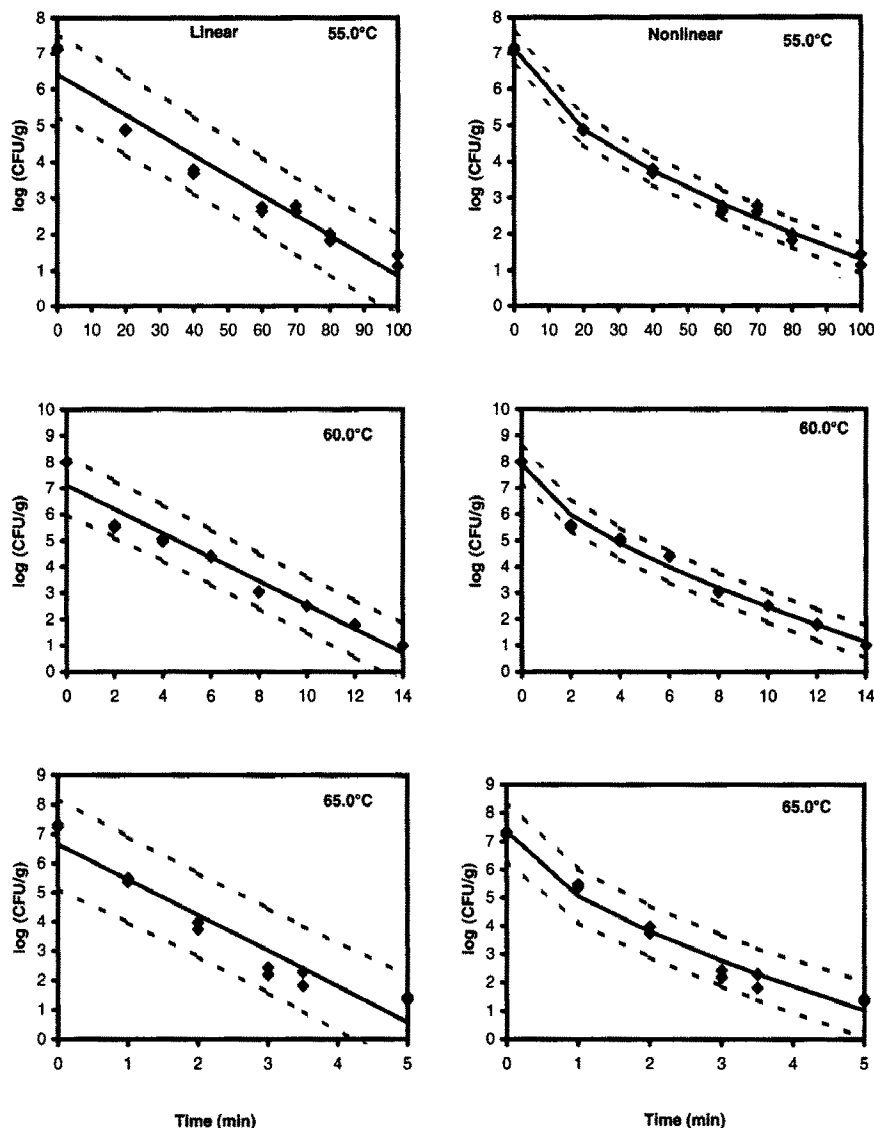
FIGURE 3. *Prediction of survivor curves of* E. coli *O157:H7 by linear and nonlinear models at selected temperatures. Broken lines are upper and lower prediction limits (95% confidence level).*
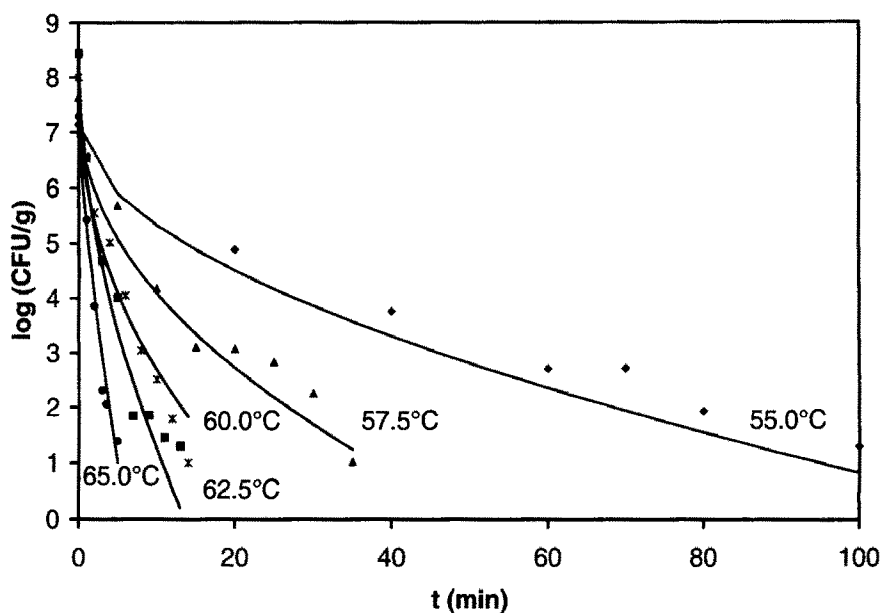

FIGURE 4. *Survivor curves at different temperatures predicted by the new models using equations 5, 7, and 8.*

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ditions. These survivor curves are similar in shape to ones observed by Juneja et al. *(7)*, although both convex concave survivor curves of *E. coli* O157:H7 in ground f have been reported by Ahmed et al. *(1)*, Jackson et al. , and Line et al. *(8)*.

The *D*-values of *E. coli* O157:H7 in 93% lean ground ef calculated from the linear model are listed in Table 1. ie *z*-value of *E. coli* O157:H7 is 7.09 ± 0.37°C in 93% in ground beef. This value is comparable in range to the values reported by Juneja et al. *(7)* but higher than the *z*-lues reported by Ahmed et al. *(1)*, Jackson et al. *(6)*, and ine et al. *(8)*, where only one strain of *E. coli* O157:H7 'as used. On average, the initial log cell concentrations redicted by the linear model were lower than the original alues by 1.01 with a standard error of ±0.20 ($P < 0.001$). With a significant underestimation in the initial bacterial :ell concentration, the thermal process calculation based on D-values under isothermal conditions is not accurate. In an example demonstrated in Figure 2, ground beef inoculated with an initial $\log_{10}$ (CFU/g) of 7.16 was heated at 55°C for up to 100 min. In this experiment, a four-dimensional (4-D) reduction of the viable cells of *E. coli* O157:H7 required only 50.08 min of exposure to 55°C (determined by interpolation of the experimental data). If the thermal process calculation is based on equation 1 using the initial cell concentration and the *D*-value (18.11 min), a 4-D reduction of the cell load would require 4×18.11, or 72.44 min. Therefore, using the *D*-values and *z*-values predicted by the linear model would lead to substantially inaccurate thermal process calculations since underestimation occurs at all temperatures.

Nonlinear regression using the new model provides much better predictions of survivor curves, as illustrated in both Table 1 and Figure 3. The correlation coefficient (R^2) of the nonlinear model is consistently higher than its linear counterpart ($P < 0.001$). According to this model, the first term *A* on the right-hand side of equation 7 should predict the initial cell concentration. No statistical difference was found between the original initial cell concentrations and the values predicted by the nonlinear model ($P = 0.9676$). More accurate thermal process calculations can be achieved using the nonlinear model. In the heating experiment demonstrated in Figure 2, the 4-D inactivation time predicted by the new model is 51.12 min, very close to the experimentally determined value of 50.08 min.

The basic assumption of the proposed nonlinear model is that the rate of thermal inactivation is both a function of the surviving cell concentration and the heating time. Numerically, the rate constant of thermal inactivation (k) increases with temperature at a rate of 0.261/°C within the test temperature ranges (equation 8, $R^2 = 0.950$). All the time indexes (*a*) are negative (Table 2), which is a clear indication of the upward concavity of the survivor curves.

In designing a thermal process, the lethality calculation is traditionally based on the experimentally determined *D*-

values and *z*-values and the time-temperature history. The parameters of the new model can also be correlated with heating temperature to obtain general models for thermal process calculations. Using linear regression, the mean time index (*a*) at each temperature can be expressed as a polynomial function of the temperatures (equation 9, $R^2 = 0.878$). Using the initial cell counts and parameters predicted by equations 8 and 9, excellent agreement between the new model and experimental data can be achieved (Fig. 4).

$$k = -13.73 + 0.261T \qquad (8)$$

$$a = -86.23 + 4.70T - 0.0855T^2 + 0.000515T^3 \quad (9)$$

In conclusion, the proposed new nonlinear model can describe the thermal inactivation process of *E. coli* O157:H7 in ground beef more accurately than the traditional linear model. Therefore, it can be a potential alternative to first-order inactivation kinetics. Since it can provide more accurate calculations, the severity of thermal processing can be reduced, and products with higher quality may be produced. The applicability will be validated with more microorganisms and experiments currently being been planned to investigate this observation.

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